

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

FILE

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/445, 31/405	A1	(11) International Publication Number: WO 96/24353
		(43) International Publication Date: 15 August 1996 (15.08.96)

(21) International Application Number: **PCT/US96/01737**(22) International Filing Date: **8 February 1996 (08.02.96)**(30) Priority Data:
08/387,056 **10 February 1995 (10.02.95)** **US**

(60) Parent Application or Grant

(63) Related by Continuation

US

Filed on

08/387,056 (CON)**10 February 1995 (10.02.95)**(71) Applicant (for all designated States except US): **ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).**

(72) Inventors; and

(75) Inventors/Applicants (for US only): **JOHNSON, Kirk, W. [US/US]; 31 Triple Crown Lane, Camby, IN 46113 (US). PHEBUS, Lee, A. [US/US]; 1744 West 1000 North, Fountaintown, IN 46130 (US).**(74) Agents: **GAYLO, Paul, J. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).**(81) Designated States: **AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).****Published***With international search report.*(54) Title: **METHODS OF TREATING OR PREVENTING PSYCHIATRIC DISORDERS**

(57) Abstract

This invention provides methods for the treatment or prevention of psychiatric disorders which comprises administering to a mammal in need thereof a combination of a tachykinin receptor antagonist and either a serotonin agonist or a selective serotonin reuptake inhibitor. This administration may be concurrent or sequential, with either of the two activities being administered first. The psychiatric disorders which may be treated by the methods of the present invention include panic disorder, panic attack, depression, anxiety, obsessive-compulsive disorder, post-traumatic stress disorder, borderline personality disorder, agoraphobia, attention deficit hyperactivity disorder, disruptive behavior disorder, intermittent explosive disorder, and borderline personality disorder.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

- 1 -

METHODS OF TREATING OR PREVENTING
PSYCHIATRIC DISORDERS

5

Since the discovery of serotonin (5-hydroxytryptamine, 5-HT) over four decades ago, the cumulative results of many diverse studies have indicated that serotonin plays a significant role in the functioning of the mammalian body, both in the central nervous system and in peripheral systems as well. Morphological studies of the central nervous system have shown that serotonergic neurons, which originate in the brain stem, form a very diffuse system that projects to most areas of the brain and spinal cord. R.A. O'Brien, Serotonin in Mental Abnormalities, 1:41 (1978); H.W.M. Steinbusch, HANDBOOK OF CHEMICAL NEUROANATOMY, Volume 3, Part II, 68 (1984); N.E. Anden, et al., Acta Physiologica Scandinavia, 67:313 (1966). These studies have been complemented by biochemical evidence that indicates large concentrations of 5-HT exist in the brain and spinal cord. H.W.M. Steinbusch, supra.

With such a diffuse system, it is not surprising that 5-HT has been implicated as being involved in the expression of a number of behaviors, physiological responses, and diseases which originate in the central nervous system. These include such diverse areas as sleeping, eating, perceiving pain, controlling body temperature, controlling blood pressure, depression, schizophrenia, and other bodily states. R.W. Fuller, BIOLOGY OF SEROTONERGIC TRANSMISSION, 221 (1982); D.J. Boullin, SEROTONIN IN MENTAL ABNORMALITIES 1:316 (1978); J. Barchas, et al., Serotonin and Behavior, (1973).

Serotonin plays an important role in peripheral systems as well. For example, approximately 90% of the

- 2 -

body's serotonin is synthesized in the gastrointestinal system, and serotonin has been found to mediate a variety of contractile, secretory, and electrophysiologic effects in this system. Serotonin may be taken up by the platelets and, upon platelet aggregation, be released such that the cardiovascular system provides another example of a peripheral network that is very sensitive to serotonin. Given the broad distribution of serotonin within the body, it is understandable that tremendous interest in drugs that affect serotonergic systems exists. In particular, receptor-specific agonists and antagonists are of interest for the treatment of a wide range of disorders, including anxiety, depression, hypertension, migraine, compulsive disorders, schizophrenia, autism, neurodegenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, and cancer chemotherapy-induced vomiting. M.D. Gershon, et al., THE PERIPHERAL ACTIONS OF 5-HYDROXYTRYPTAMINE, 246 (1989); P.R. Saxena, et al., Journal of Cardiovascular Pharmacology, 15:Supplement 7 (1990).

Serotonin produces its effects on cellular physiology by binding to specialized receptors on the cell surface. It is now recognized that multiple types of receptors exist for many neurotransmitters and hormones, including serotonin. The existence of multiple, structurally distinct serotonin receptors has provided the possibility that subtype-selective pharmacologic agents can be produced. The development of such compounds could result in new and increasingly selective therapeutic agents with fewer side effects, since activation of individual receptor subtypes may function to affect specific actions of the different parts of the central and/or peripheral serotonergic systems.

An example of such specificity can be demonstrated by using the vascular system as an example. In certain blood vessels, stimulation of 5-HT₁-like receptors on the endothelial cells produces vasodilation

- 3 -

while stimulation of 5-HT₂ receptors on the smooth muscle cells produces vasoconstriction.

Currently, the major classes of serotonin receptors (5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆, and 5-HT₇) contain some fourteen to eighteen separate receptors that have been formally classified based on their pharmacological or structural differences. [For an excellent review of the pharmacological effects and clinical implications of the various 5-HT receptor types, see Glennon, et al., Neuroscience and Behavioral Reviews, 14:35 (1990).]

Tachykinins are a family of peptides which share a common amidated carboxy terminal sequence. Substance P was the first peptide of this family to be isolated, although its purification and the determination of its primary sequence did not occur until the early 1970's.

Between 1983 and 1984 several groups reported the isolation of two novel mammalian tachykinins, now termed neurokinin A (also known as substance K, neuromedin L, and neurokinin α), and neurokinin B (also known as neuromedin K and neurokinin β). See, J.E. Maggio, Peptides, 6 (Supplement 3):237-243 (1985) for a review of these discoveries.

Tachykinins are widely distributed in both the central and peripheral nervous systems, are released from nerves, and exert a variety of biological actions, which, in most cases, depend upon activation of specific receptors expressed on the membrane of target cells. Tachykinins are also produced by a number of non-neural tissues.

The mammalian tachykinins substance P, neurokinin A, and neurokinin B act through three major receptor subtypes, denoted as NK-1, NK-2, and NK-3, respectively. These receptors are present in a variety of organs.

Substance P is believed inter alia to be involved in the neurotransmission of pain sensations,

- 4 -

including the pain associated with migraine headaches and with arthritis. These peptides have also been implicated in gastrointestinal disorders and diseases of the gastrointestinal tract such as inflammatory bowel disease. 5 Tachykinins have also been implicated as playing a role in numerous other maladies, as discussed infra.

Tachykinins play a major role in mediating the sensation and transmission of pain or nociception, especially migraine headaches. see, e.g., S.L. Sheppard, 10 et al., British Journal of Pharmacology, 108:11-20 (1993); S.M. Moussaoui, et al., European Journal of Pharmacology, 238:421-424 (1993); and W.S. Lee, et al., British Journal of Pharmacology, 112:920-924 (1994).

In view of the wide number of clinical maladies 15 associated with an excess of tachykinins, the development of tachykinin receptor antagonists will serve to control these clinical conditions. The earliest tachykinin receptor antagonists were peptide derivatives. These antagonists proved to be of limited pharmaceutical utility 20 because of their metabolic instability.

Recent publications have described novel classes of non-peptidyl tachykinin receptor antagonists which generally have greater oral bioavailability and metabolic stability than the earlier classes of tachykinin receptor 25 antagonists. Examples of such newer non-peptidyl tachykinin receptor antagonists are found in European Patent Publication 591,040 A1, published April 6, 1994; Patent Cooperation Treaty publication WO 94/01402, published January 20, 1994; Patent Cooperation Treaty 30 publication WO 94/04494, published March 3, 1994; Patent Cooperation Treaty publication WO 94/07843, published April 14, 1994; and Patent Cooperation Treaty publication WO 93/01169, published January 21, 1993.

Because of the current dissatisfaction of the 35 currently marketed treatments for the psychiatric disorders

- 5 -

discussed below within the affected population, there exists a need for a more efficacious and safe treatment.

This invention provides methods for the
5 treatment or prevention of a psychiatric disorder in a mammal which comprise administering to a mammal in need thereof an effective amount of a composition having both tachykinin receptor antagonist activity and either
10 serotonin agonist activity or activity as a selective serotonin reuptake inhibitor.

In particular, the present invention provides methods for treating persons afflicted with, or with a heightened risk of contracting, one or more disorders selected from the group consisting of panic disorder, panic
15 attack, depression, anxiety, bulimia nervosa, obsessive-compulsive disorder, premenstrual dysphoric disorder, substance abuse, substance dependence, agoraphobia, post-traumatic stress disorder, dementia of Alzheimer's type, social phobia, attention deficit hyperactivity disorder,
20 disruptive behavior disorder, intermittent explosive disorder, borderline personality disorder, chronic fatigue syndrome, premature ejaculation, and depression and behavioral problems associated with head injury, mental retardation or stroke.

This invention further provides methods for the
25 treatment or prevention of a psychiatric disorder in a mammal which comprise the sequential administration to a mammal in need thereof a composition having serotonin agonist activity (or selective serotonin reuptake inhibitor
30 activity) followed by the administration of a composition having tachykinin receptor antagonist activity.

This invention also provides methods for the
treatment or prevention of a psychiatric disorder in a
mammal which comprise the sequential administration to a
35 mammal in need thereof a composition having tachykinin receptor antagonist activity followed by the administration

- 6 -

of a composition having serotonin agonist activity (or selective serotonin reuptake inhibitor activity).

The terms and abbreviations used in the instant preparations and examples have their normal meanings unless otherwise designated. For example "°C" refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "L" means liter or liters; "M" refers to molar or molarity; "MS" refers to mass spectrometry; "IR" refers to infrared spectroscopy; and "NMR" refers to nuclear magnetic resonance spectroscopy.

Many serotonin binding receptors have been identified. These receptors are generally grouped into seven classes on the basis of their structure and the pharmacology of the receptor as determined by the binding efficiency and drug-related characteristics of numerous serotonin receptor-binding compounds. In some of the groups several subtypes have been identified. [For a relatively recent review of 5-hydroxytryptamine receptors, see, E. Zifa and G. Fillion, Pharmacological Reviews, 44:401-458 (1992); D. Hoyer, et al., Pharmacological Reviews, 46:157-203 (1994).] Table I, infra, lists the seven classes of serotonin receptors as well as several known subtypes. This table also provides the physiological distribution of these receptors as well as biological responses mediated by the receptor class or subtype, if any such response is known. This table is derived from D. Hoyer, et al., "VII. International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin)", Pharmacological Reviews, 46:157-203 (1994), a publication of the Serotonin Club Receptor Nomenclature Committee of the IUPHAR Committee for Receptor Nomenclature.

Table I

Receptor Type	Subtype	Location	Response
5-HT ₁	5-HT _{1A}	Neuronal, mainly in CNS	Neuronal hyperpolarisation, hypotension
	5-HT _{1B}	CNS and some peripheral nerves	Inhibition of neurotransmitter release
	5-HT _{1D}	Mainly CNS	Inhibition of neurotransmitter release
	5-HT _{1E}	Only CNS	Inhibition of adenylyl cyclase
	5-HT _{1F}	Mainly CNS	Inhibition of adenylyl cyclase
	5-HT ₁ -like	Intracranial vasculature	Smooth muscle contraction
5-HT ₂	5-HT _{2A}	Vascular smooth muscle, platelets, lung, CNS, gastrointestinal tract	Vasoconstriction, platelet aggregation, bronchoconstriction
	5-HT _{2B}	Mainly peripheral, some CNS	Rat stomach fundic muscle contraction
	5-HT _{2C}	CNS (high density in choroid plexus)	upregulates phosphoinositide turnover
5-HT ₃		Peripheral and central neurones	Depolarization
5-HT ₄		Gastrointestinal tract, CNS, heart, urinary bladder	Activation of acetylcholine release in gut, tachycardia, upregulates cAMP in CNS neurones
5-HT ₅	5-HT _{5A}	CNS	Not known
	5-HT _{5E}	CNS	Not known
5-HT ₆		CNS	Activation of adenylyl cyclase
5-HT ₇		CNS	Activation of adenylyl cyclase

- 8 -

The Hoyer, et al., reference describes for each class or subtype one or more compounds which have efficacy as antagonists or agonists for the receptor.

5 The 5-HT₁ family includes subtypes which can be grouped together based on the absence of introns in the cloned genes, a common G-coupled protein transduction system (inhibition of adenylate cyclase), and similar operational characteristics. The 5-HT₁ family of
10 inhibitory receptors includes subtypes A, B, D, E, and F. The 5-HT₁ G protein-linked receptors general inhibit the production of cyclic adenosine monophosphate (cAMP), while the 5-HT₂ G protein linked receptors stimulate
15 phosphoinositol hydrolysis.

15 The 5-HT_{1A} receptor was the first cloned human serotonin receptor. Activated 5-HT_{1A} receptors expressed in HeLa cells inhibit forskolin-stimulated adenylate cyclase activity. The 5-HT_{1D} receptor was originally identified in
20 bovine brain membrane by Heuring and Peroutka. R.E. Heuring and S.J. Peroutka, Journal of Neuroscience, 7:894-903 (1987). The 5-HT_{1D} receptors are the most common 5-HT
25 receptor subtype in the human brain and may be identical to the 5-HT₁-like receptor in the cranial vasculature. S.D. Silberstein, Headache, 34:408-417 (1994). Sumatriptan and
30 the ergot alkaloids have high affinity for both the human 5-HT_{1D} and the 5-HT_{1B} receptors. Id.

30 The 5-HT_{1F} subtype of receptor has low affinity for 5-carboxamidotryptamine (5-CT) unlike the other 5-HT receptors, except for the 5-HT_{1E} subtype. Unlike the
35 5-HT_{1E} receptors, however, the 5-HT_{1F} receptors do show affinity for sumatriptan.

35 The biological efficacy of a compound believed to be effective as a serotonin agonist may be confirmed by first employing an initial screening assay which rapidly
and accurately measures the binding of the test compound to one or more serotonin receptors. Once the binding of the

- 9 -

test compound to one or more serotonin receptors is established, the in vivo activity of the test compound on the receptor is established. Assays useful for evaluating serotonin agonists are well known in the art. See, e.g., E. Zifa and G. Fillion, supra; D. Hoyer, et al., supra, and the references cited therein.

Serotonin Receptor Binding Activity

10 Binding to the 5-HT_{1F} receptor.

The ability of a compound to bind to a serotonin receptor was measured using standard procedures. For example, the ability of a compound to bind to the 5-HT_{1F} receptor subtype was performed essentially as described in
15 N. Adham, et al., Proceedings of the National Academy of Sciences (USA), 90:408-412 (1993).

The cloned 5-HT_{1F} receptor was expressed in stably transfected LM(tk⁻) cells. Membrane preparations were made by growing these transfected cell lines to
20 confluency. The cells were washed twice with phosphate-buffered saline, scraped into 5 ml of ice-cold phosphate-buffered saline, and centrifuged at 200 x g for about five minutes at 4°C. The pellet was resuspended in 2.5 ml of cold Tris buffer (20 mM Tris·HCl, pH 7.4 at 23°C, 5 mM
25 EDTA) and homogenized. The lysate was centrifuged at 200 x g for about five minutes at 4°C to pellet large fragments. The supernatant was then centrifuged at 40,000 x g for about 20 minutes at 4°C. The membranes were washed once in the homogenization buffer and resuspended in
30 25 mM glycylglycine buffer, pH 7.6 at 23°C.

Radioligand binding studies were performed using [³H]5-HT (20-30 Ci/mmol). Competition experiments were done by using various concentrations of drug and 4.5-5.5 nM [³H]5-HT. Nonspecific binding was defined by 10 μM 5-HT.
35 Binding data were analyzed by nonlinear-regression

- 10 -

analysis. IC₅₀ values were converted to K_i values using the Cheng-Prusoff equation.

For comparison purposes, the binding affinities of compounds for various serotonin receptors may be determined essentially as described above except that different cloned receptors are employed in place of the 5-HT_{1F} receptor clone employed therein.

10 Serotonin Agonist Activity

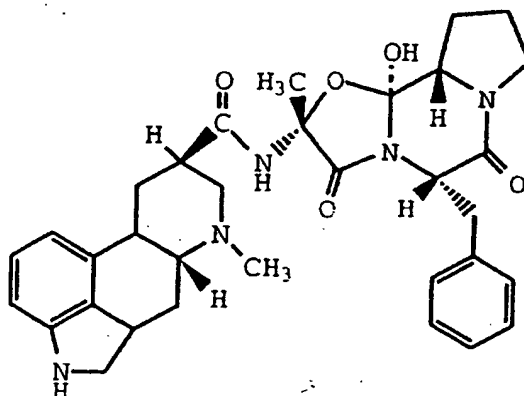
Adenylate Cyclase Activity.

Adenylate cyclase activity was determined in initial experiments in LM(tk-) cells, using standard techniques. See, e.g., N. Adham, et al., supra.; R.L. Weinshank, et al., Proceedings of the National Academy of Sciences (USA), 89:3630-3634 (1992), and the references cited therein.

Intracellular levels of cAMP were measured using the clonally derived cell line described above. Cells were preincubated for about 20 minutes at 37°C in 5% carbon dioxide, in Dulbecco's modified Eagle's medium containing 10 mM HEPES, 5 mM theophylline, and 10 μM pargyline. Varying concentrations of the test compounds were added to this medium to determine inhibition of forskolin-stimulated adenylate cyclase.

Some compounds that bind serotonin receptors show no receptor selectivity, i.e. they bind different receptor subtypes with comparable affinity. One example of such a non-selective serotonin receptor binding compound is dihydroergotamine, a compound having the structure

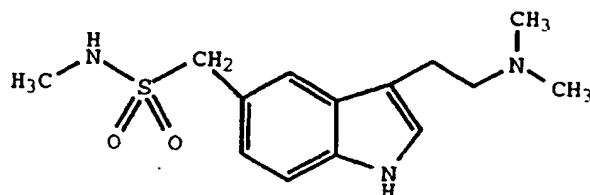
- 11 -



and the chemical name, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)ergotaman-3',6',18'-trione. This compound
 5 is commercially available [as the mesylate salt] or may be prepared as described in Stoll and Hofmann, Helv. Chimica Acta, 26:2070 (1943).

A compound having a high affinity for one (or a few) receptor subtype and low affinity for other receptor
 10 subtypes using studies analogous to the binding assays supra, is considered to be subtype-selective. Such compounds are especially preferred in the methods of the present invention.

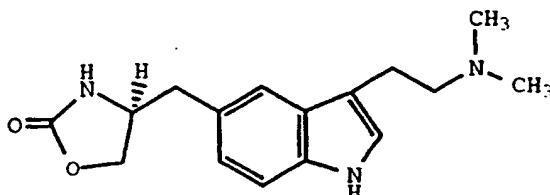
One example of such a compound is sumatriptan, a
 15 compound having the structure



and the chemical name, 3-[2-(dimethylamino)ethyl]-N-methyl-1H-indole-5-methanesulfonamide. This compound is
 20 commercially available or may be prepared as described in United States Patent 5,037,845, issued August 6, 1991, which is herein incorporated by reference. Sumatriptan is selective for the 5-HT₁ receptor subtypes.

- 12 -

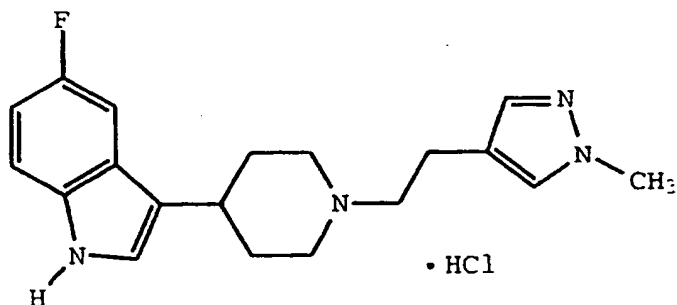
An additional serotonin agonist which is specific for the 5-HT₁ class of receptors is a compound of the structure



5

having the designation 311C90 and the chemical name (S)-4-[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]-2-oxazolidinone. This compound may be synthesized as described in Patent Cooperation Treaty Publication WO 91/18897, published December 12, 1991. Unlike sumatriptan, 311C90 is believed capable of crossing the blood-brain barrier. Scrip, September 7, 1994.

Especially preferred serotonin agonists employed in the methods of this invention are those compounds with a high affinity for the 5-HT_{1F} subtype of receptor. One such class of compounds is typified by the compound



20

having the chemical name 5-fluoro-3-[1-[2-[1-methyl-1H-pyrazol-4-yl]ethyl]-4-piperidinyl]-1H-indole hydrochloride. This compound may be prepared as described in co-pending United States patent application 08/318,329, filed October 5, 1994.

25

- 13 -

Essentially, to a solution of 2.0 g (9.2 mmol) 5-fluoro-3-(4-piperidinyl)-1H-indole in 50 ml dimethylformamide were added 2.65 g (0.025 mole) sodium carbonate followed by 1.87 g (9.2 mmol) 1-methyl-4-(2-methanesulfonyloxyethyl)-1H-pyrazole. The resulting mixture was heated at 100°C for 18 hours under nitrogen. The dimethylformamide was distilled under reduced pressure and the resulting residue was partitioned between water and dichloromethane. The dichloromethane phase was separated, washed sequentially with water and saturated aqueous sodium chloride solution and then dried over sodium sulfate to give 4.0 g of a brown oil. The brown oil was chromatographed over silica gel, eluting with 95:5 dichloromethane:methanol. Fractions shown to contain product were combined and concentrated under reduced pressure to give 5-fluoro-3-[1-[2-[1-methyl-1H-pyrazol-4-yl]ethyl]-4-piperidinyl]-1H-indole as a yellow oil. The oil was dissolved in a minimal volume of methanol and to it were added 1.21 ml (0.006 mole) 5N hydrochloric acid. To the resulting solution was added ethyl acetate to the point of incipient precipitation. The solid recovered was recrystallized from methanol/ethyl acetate to give 1.61 g (51.1%) of the title compound as an off-white solid.

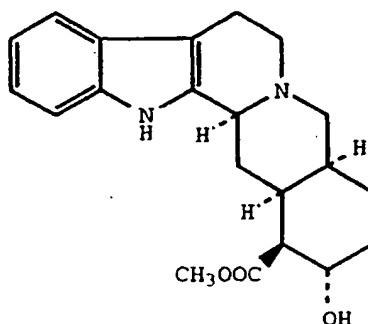
The starting materials described herein are either commercially available or may be synthesized from commercially available materials using known methods.

Some additional classical serotonin agonists which are frequently employed are:

30

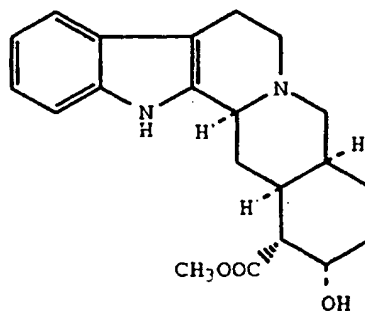
(a) Rauwolscine -- a compound of the formula

- 14 -



having the chemical name 17 α -hydroxy-20 α -yohimban-16 β -
carboxylic acid methyl ester. This compound, also known as
5 α -yohimbine, can be prepared as described in Töke, et al.,
Journal of Organic Chemistry, 38:2496 (1973) or can be
purchased commercially from many sources.

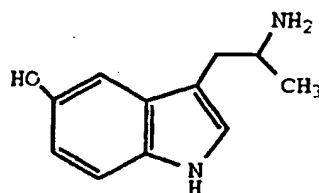
(b) Yohimbine -- a compound also known as allo-
10 yohimbine having the formula



with the chemical name 17-hydroxyyohimban-16-carboxylic
15 acid methyl ester. This compound, which is available from
commercial sources, can also be synthesized as described in
Töke, et al., supra.

(c) α -Methyl-5-hydroxytryptamine -- a compound
20 of the formula

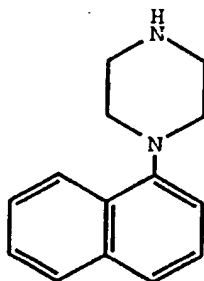
- 15 -



having the chemical name 3-(2-aminopropyl)-1H-indol-5-ol,
which is available from commercial sources.

5

(d) 1-(1-Naphthyl)piperazine -- a compound of
the formula

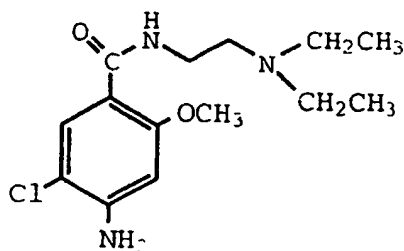


10

which is described in U.S. Patent 4,520,024, issued May 28,
1985, which is herein incorporated by reference.

(e) Metoclopramide -- a compound of the formula

15



having the chemical name 4-amino-5-chloro-N-[(2-
diethylamino)ethyl]-2-methoxybenzamide, which is described
in United States Patent 3,177,252, which is herein
incorporated by reference.

20

- 16 -

The above groups of compounds are only illustrative of the serotonin receptor agonists which are currently under development or are frequently employed in serotonin receptor studies. This listing of groups of compounds is not meant to be comprehensive, the methods of the present invention may employ any serotonin receptor agonist and is not limited to any particular class of compound.

10

In addition to the methods supra employing serotonin agonists, this invention also encompasses methods for the treatment or prevention of a psychiatric disorder in a mammal which comprise administering to a mammal in need thereof an effective amount of a composition having both tachykinin receptor antagonist activity and selective serotonin reuptake inhibition activity.

This invention further provides methods for the treatment or prevention of a psychiatric disorder in a mammal which comprise the sequential administration to a mammal in need thereof a composition having selective serotonin reuptake inhibition activity followed by the administration of a composition having tachykinin receptor antagonist activity.

This invention also provides methods for the treatment or prevention of a psychiatric disorder in a mammal which comprise the sequential administration to a mammal in need thereof a composition having tachykinin receptor antagonist activity followed by the administration of a composition having selective serotonin reuptake inhibition activity.

The selective serotonin reuptake inhibitors (SSRI's) are a series of compounds which selectively inhibit the serotonin transporter on membranes of serotonin neurons. These uptake inhibitors increase the concentration of serotonin within the synaptic cleft by

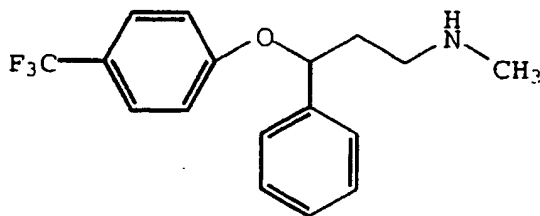
- 17 -

clocking its removal via the membrane transporter.

Inhibitors of serotonin uptake increase serotonin action on postsynaptic receptors on target neuron and increase serotonergic neurotransmission, resulting in functional
5 cnosequences that are mostly subtle, i.e., not detectable by gross observation, but are detectable by various specific techniques.

For instance, serotonin uptake inhibitors reduce aggressive behavior, decrease food uptake, decrease alcohol
10 drinking in reats, decrease rapid-eye-movement sleep, potentiate morphine analgesia, and the like. R.W. Fuller, Journal of Clinical Psychiatry, 53:35-45 (1992). Serotonin uptake inhibitors are used clinically in the treatment of mental depression, bulimia, and obsessive-compulsive
15 disorder. They are also reported to be effective as appetite suppressant drugs in the treatment of obesity, in borderline personality disorder, trichotillomania, panic disorder, and attention deficit hyperactivity disorder. See, e.g., R.W. Fuller, Advances in Biosciences, 85:255-270
20 (1992). In addition, serotonin uptake inhibitors have been reported to have therapeutic benefit in premenstrual syndrome, diabetic neuropathy, certain non-cognitive symptoms of Alzheimer's Disease, chronic pain, and in postanoxic intention myoclonus. Id.

25 One such compound is fluoxetine, a compound having the structure

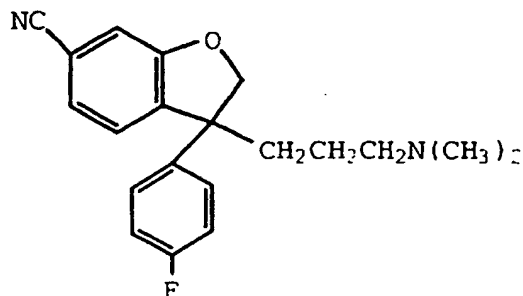


30 and the chemical name N-methyl-3-(4-trifluoromethylphenoxy)-3-phenylpropylamine. This compound

- 18 -

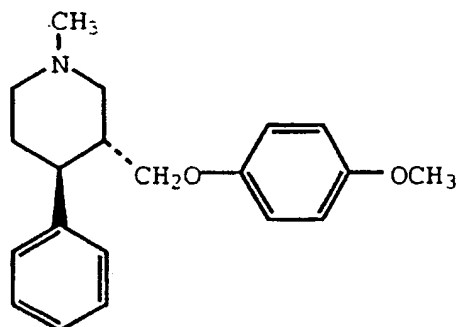
is prepared as described in United States Patent 4,314,081 which is herein incorporated by reference.

Another selective serotonin reuptake inhibitor which may be employed in the methods of the present invention is citalopram, a compound having the structure



and the chemical name 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile. This compound may be prepared as described in United States Patent 4,136,193, the entire contents of which are herein incorporated by reference.

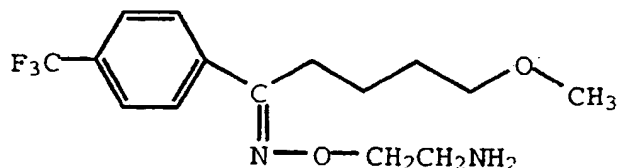
Another compound belonging to this class of therapeutics is femoxetine, a compound having the structure



and the chemical name 2-(1,3,4-oxadiazol-2-yl)phenol. This compound may be prepared as described in United States Patent 3,912,743, the entire contents of which are herein incorporated by reference.

- 19 -

Another SSRI which may be employed in the methods of the present invention is fluvoxamine, a compound having the structure

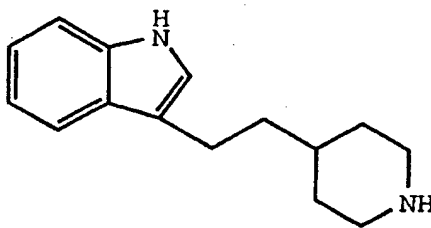


5

and the chemical name 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone O-(2-aminoethyl)oxime. This compound may be prepared as described in United States Patent 4,085,225, the entire contents of which are herein incorporated by reference.

10

Another compound belonging to this class of therapeutics is indalpine, a compound having the structure



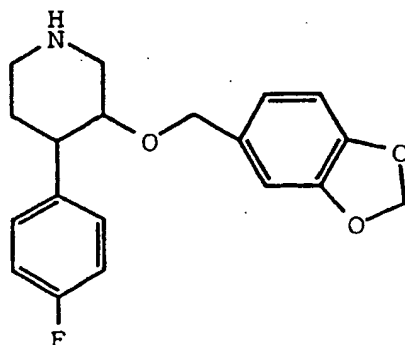
15

and the chemical name 3-[2-(4-piperidinyl)ethyl]-1H-indole. This compound may be prepared as described in United States Patent 4,064,255, the entire contents of which are herein incorporated by reference.

20

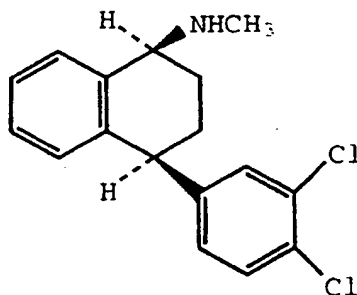
Another such compound is paroxetine, a compound having the structure

- 20 -



and the chemical name trans-(-)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine. This compound
5 is prepared as described in United States Patents 3,912,743 and 4,007,196, the entire contents of which are herein incorporated by reference.

Sertraline is another SSRI which may be employed in the methods of the present invention. This compound,
10 having the chemical name (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine, has the following structure.

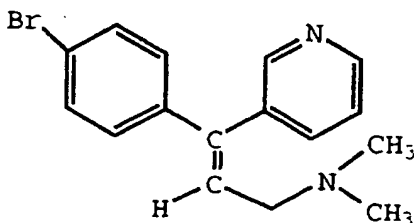


15

Sertraline may be prepared as described in United States Patent 4,536,518, the entire contents of which are herein incorporated by reference.

An additional SSRI which may be employed in the
20 methods of the present invention is zimeldine, a compound of the structure

- 21 -

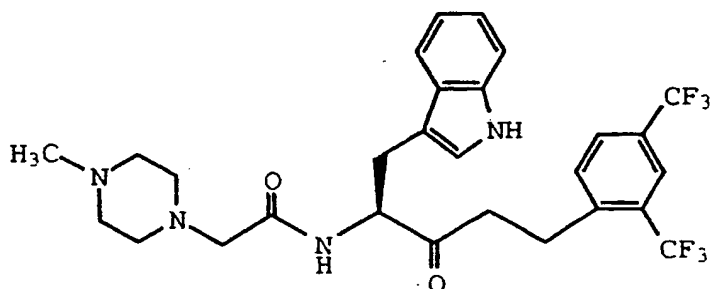


having the chemical name (Z)-3-(4-bromophenyl)-N,N-dimethyl-3-(3-pyridinyl)-2-propen-1-amine. This compound
5 may be prepared as described in United States Patent 3,928,369, the entire contents of which are herein incorporated by reference.

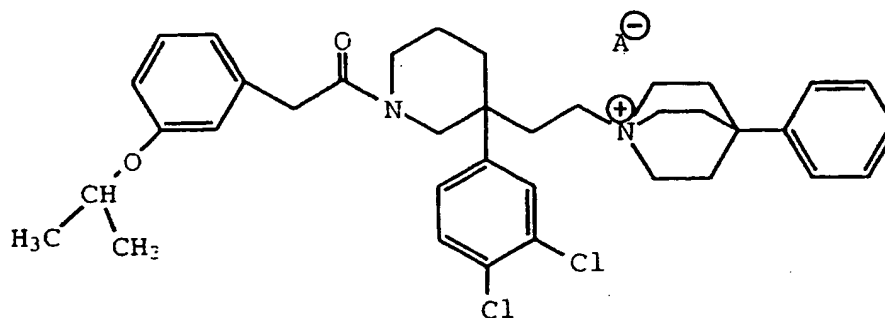
The above groups of compounds are only illustrative of the selective serotonin reuptake inhibitors
10 which are currently under development or are frequently employed in serotonin receptor studies. This listing of groups of compounds is not meant to be comprehensive, the methods of the present invention may employ any selective serotonin reuptake inhibitors and is not limited to any
15 particular class of compound.

The methods of the present invention, in addition to the serotonin agonists and selective serotonin reuptake inhibitors, examples of which are described above,
20 also employ various tachykinin receptors. In recent publications many different groups of non-peptidyl tachykinin receptor antagonists have been described.

Patent Cooperation Treaty publication WO 94/01402, published January 20, 1994, describes a series of
25 compounds best typified by the following compound.

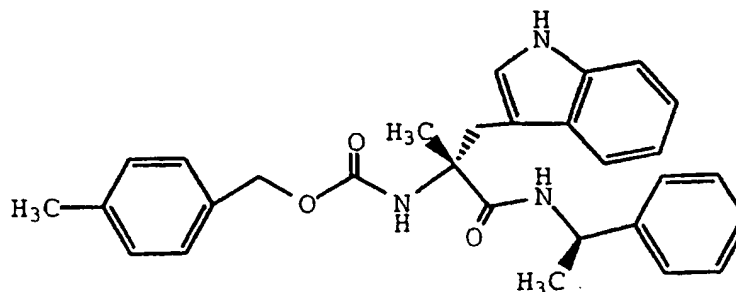


European Patent Publication 591,040 A1,
published April 6, 1994 describes a series of compounds
5 typified by the following compound:



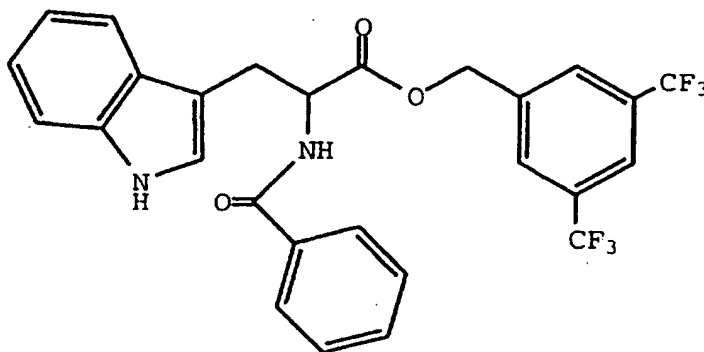
where A^{\ominus} is a pharmaceutically acceptable anion.

Patent Cooperation Treaty publication WO 94/04494, published March 3, 1994, describes a series of compounds typified by the following compound.



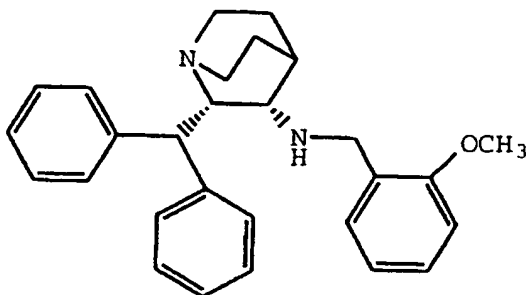
- 23 -

Patent Cooperation Treaty publication WO 93/01169, published January 21, 1993, describes a series of compounds typified by the following compound.



5

Another group of tachykinin receptor antagonists is characterized by the compound of the formula:



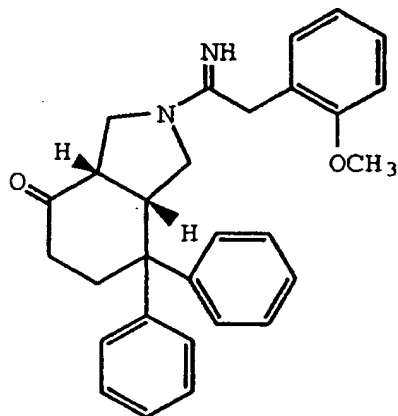
10

having the designation (±)-CP 96345. These compounds and their syntheses are described in E.J. Warawa, et al., Journal of Medicinal Chemistry, 18:357 (1975).

15

Yet another group of tachykinin receptor antagonists is characterized by the compound of the formula:

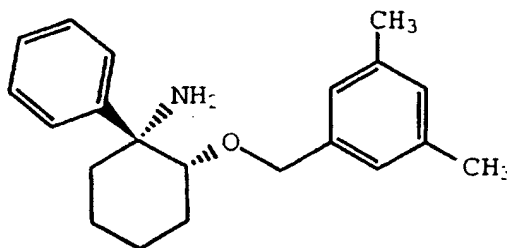
- 24 -



having the designation RP 67580. These compounds and their
syntheses are described in C. Garret, et al., Proceedings
5 of the National Academy of Sciences (USA), 88:10208-10211
(1991) and the references cited therein.

Patent Cooperation Treaty publication WO
94/07843 describes a series of cyclohexylamine derivatives
typified by the following compound

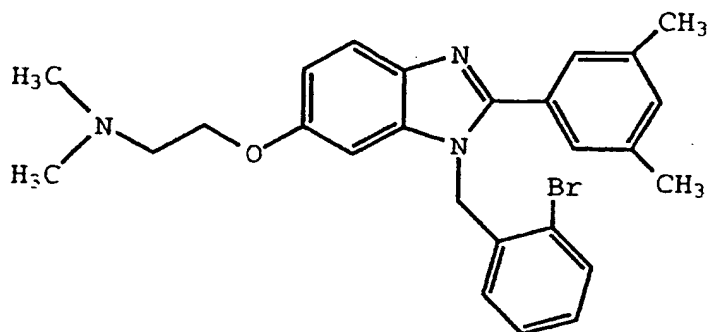
10



which are useful as tachykinin receptor antagonists.

Another group of compounds useful as tachykinin
15 receptor antagonists is typified by the following compound.

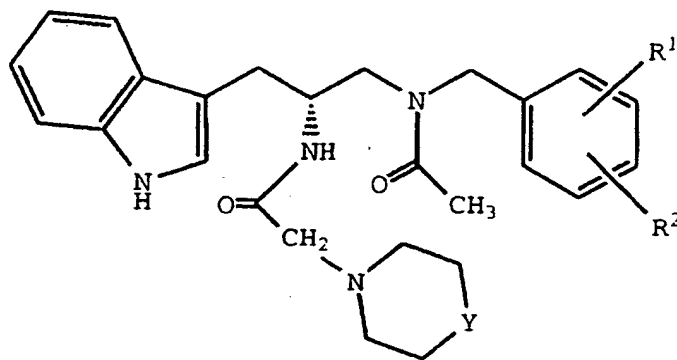
- 25 -



The synthesis of these compounds is described in co-pending United States patent application 08/235401, filed April 29, 1994.

The above groups of compounds are only illustrative of the tachykinin receptor antagonists which are currently under development. This listing of groups of compounds is not meant to be comprehensive, the methods of the present invention may employ any tachykinin receptor antagonist and is not limited to any particular class of compound.

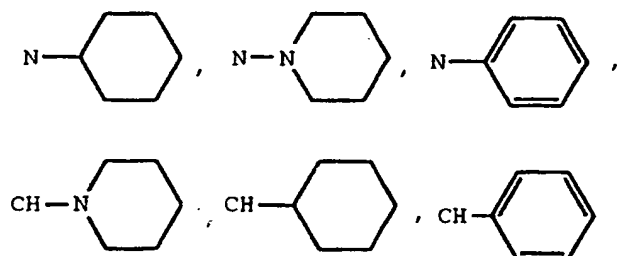
A most preferred class of tachykinin receptor antagonists are those compounds of the following structure



where R^1 and R^2 are independently selected from the group consisting of hydrogen, methyl, methoxy, chloro, and trifluoromethyl, with the proviso that no more than one of R^1 and R^2 can be hydrogen; and

- 26 -

Y is

N-R^a, or CH-NR^bR^c,

where R^a, R^b, and R^c are independently selected from the group consisting of hydrogen and C₁-C₆ alkyl;

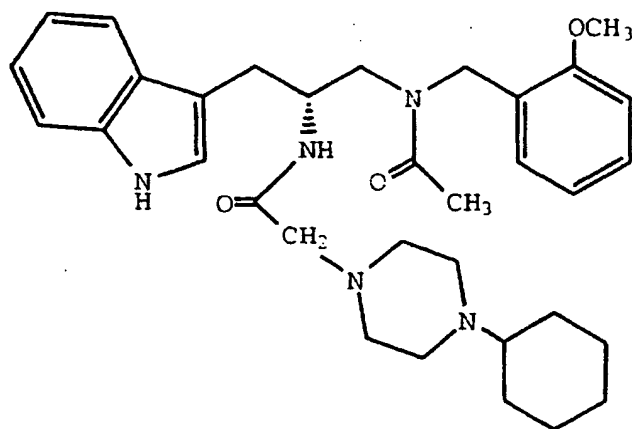
10

or a pharmaceutically acceptable salt or solvate thereof. The synthesis of these compounds is described in co-pending United States Patent Application Serial Number 08/153,847, filed November 17, 1993. The syntheses of two typical compounds from this class are detailed infra.

15

Synthesis of (R)-2-[N-(2-((4-cyclohexyl)piperazin-1-yl)acetyl)amino]-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl]amino]propane

20

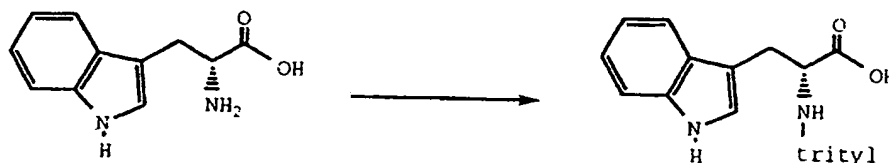


- 27 -

(a) Preparation of (R)-3-(1H-indol-3-yl)-2-(N-triphenylmethylamino)propanoic acid [N-trityltryptophan]

Tritylation

5



Chlorotrimethylsilane (70.0 ml, 0.527 mol) was added at a moderate rate to a stirred slurry of D-tryptophan (100.0 g, 0.490 mol) in anhydrous methylene chloride (800 ml) under a nitrogen atmosphere. This mixture was continuously stirred for 4.25 hours. Triethylamine (147.0 ml, 1.055 mol) was added, followed by the addition of a solution of triphenylmethyl chloride (147.0 g, 0.552 mol) in methylene chloride (400 ml) using an addition funnel. The mixture was stirred at room temperature, under a nitrogen atmosphere for at least 20 hours. The reaction was quenched by the addition of methanol (500 ml).

The solution was concentrated on a rotary evaporator to near dryness and the mixture was redissolved in methylene chloride and ethyl acetate. An aqueous work-up involving a 5% citric acid solution (2X) and brine (2X) was then performed. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness on a rotary evaporator. The solid was dissolved in hot diethyl ether followed by the addition of hexanes to promote crystallization. By this process 173.6 g (0.389 mol) of analytically pure (R)-3-(1H-indol-3-yl)-2-(N-triphenylmethylamino)propanoic acid was isolated as a white solid in two crops giving a total of 79% yield. FDMS 446 (M⁺).

- 28 -

^1H NMR ($\text{DMSO}-d_6$) δ 2.70 (m, 1H), 2.83 (m, 2H), 3.35 (m, 1H), 6.92-7.20 (m, 12H), 7.30-7.41 (m, 8H), 10.83 (s, 1H), 11.73 (br s, 1H).

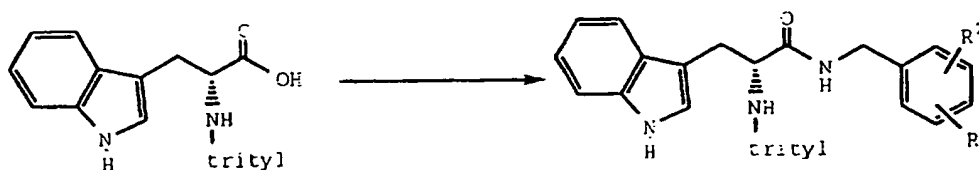
Analysis for $\text{C}_{30}\text{H}_{26}\text{N}_2\text{O}_2$:

5 Theory: C, 80.69; H, 5.87; N, 6.27.
 Found: C, 80.47; H, 5.92; N, 6.10.

(b) Preparation of (R)-3-(1H-indol-3-yl)-N-(2-methoxybenzyl)-2-(N-triphenylmethylamino)propanamide

10

Coupling



15 To a stirred solution of (R)-3-(1H-indol-3-yl)-2-(N-triphenylmethylamino)propanoic acid (179.8 g, 0.403 mol), 2-methoxybenzylamine (56.0 ml, 0.429 mol), and hydroxybenzotriazole hydrate (57.97 g, 0.429 mol) in anhydrous tetrahydrofuran (1.7 L) and anhydrous N,N-

20 dimethylformamide (500 ml) under a nitrogen atmosphere at 0°C, were added triethylamine (60.0 ml, 0.430 mol) and 1-(3-dimethylaminopropyl)-3-ethoxycarbodiimide hydrochloride (82.25 g, 0.429 mol). The mixture was allowed to warm to room temperature under a nitrogen atmosphere for at least

25 20 hours. The mixture was concentrated on a rotary evaporator and then redissolved in methylene chloride and an aqueous work-up of 5% citric acid solution (2X), saturated sodium bicarbonate solution (2X), and brine (2X) was performed. The organic layer was dried over anhydrous

30 sodium sulfate and concentrated to dryness on a rotary evaporator. The desired product was then recrystallized from hot ethyl acetate to yield 215.8 g (0.381 mol, 95%) of analytically pure material.

- 29 -

FDMS 565 (M⁺).

¹H NMR (CDCl₃) δ 2.19 (dd, J=6.4 Hz, Δν=14.4 Hz, 1H), 2.64 (d, J=6.5 Hz, 1H), 3.19 (dd, J=4.3 Hz, Δν=14.4 Hz, 1H), 3.49 (m, 1H), 3.63 (s, 3H), 3.99 (dd, J=5.4 Hz, Δν=14.2 Hz, 1H), 4.25 (dd, J=7.1 Hz, Δν=14.2 Hz, 1H), 6.64 (d, J=2.1 Hz, 1H), 6.80 (d, J=8.2 Hz, 1H), 6.91 (t, J=7.4 Hz, 1H), 7.06-7.38 (m, 21 H), 7.49 (d, J=7.9 Hz, 1H), 7.75 (s, 1H).

Analysis for C₃₈H₃₅N₃O₂:

Theory: C, 80.68; H, 6.24; N, 7.43.

Found: C, 80.65; H, 6.46; N, 7.50.

(c) Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)amino]-2-(N-triphenylmethylamino)propane

15 Reduction of Carbonyl



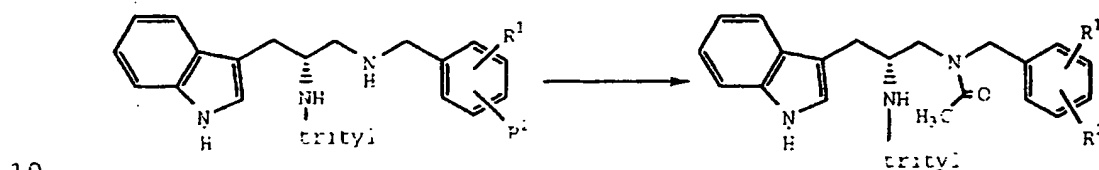
RED-AL[®], [a 3.4 M, solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene] (535 ml, 1.819 mol), dissolved in anhydrous tetrahydrofuran (400 ml) was slowly added using an addition funnel to a refluxing solution of the acylation product, (R)-3-(1H-indol-3-yl)-N-(2-methoxybenzyl)-2-(N-triphenylmethylamino)propanamide (228.6 g, 0.404 mols) produced supra, in anhydrous tetrahydrofuran (1.0 L) under a nitrogen atmosphere. The reaction mixture became a purple solution. The reaction was quenched after at least 20 hours by the slow addition of excess saturated Rochelle's salt solution (potassium sodium tartrate tetrahydrate). The organic layer was isolated, washed with brine (2X), dried over anhydrous sodium sulfate, filtered, and concentrated to an oil on a

- 30 -

rotary evaporator. No further purification was done and the product was used directly in the next step.

(d) Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)-acetylamino]-2-(N-triphenylmethylamino)propane

Acylation of Secondary Amine



To a stirring solution of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)amino]-2-(N-triphenylmethylamino)propane (0.404 mol) in anhydrous tetrahydrofuran (1.2 L) under a nitrogen atmosphere at 0°C was added triethylamine (66.5 ml, 0.477 mol) and acetic anhydride (45.0 ml, 0.477 mol). After 4 hours, the mixture was concentrated on a rotary evaporator, redissolved in methylene chloride and ethyl acetate, washed with water (2X) and brine (2X), dried over anhydrous sodium sulfate, filtered, and concentrated to a solid on a rotary evaporator. The resulting solid was dissolved in chloroform and loaded onto silica gel 60 (230-400 mesh) and eluted with a 1:1 mixture of ethyl acetate and hexanes. The product was then crystallized from an ethyl acetate/hexanes mixture. The resulting product of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylamino]-2-(N-triphenylmethylamino)propane was crystallized and isolated over three crops giving 208.97 grams (87% yield) of analytically pure material.

Analysis for $C_{40}H_{39}N_3O_2$:

Theory: C, 80.91; H, 6.62; N, 7.08.

Found: C, 81.00; H, 6.69; N, 6.94.

- 31 -

(e) Preparation of (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminopropyl]-1-[N-(2-methoxybenzyl)acetylaminopropyl]propane

5 Deprotection

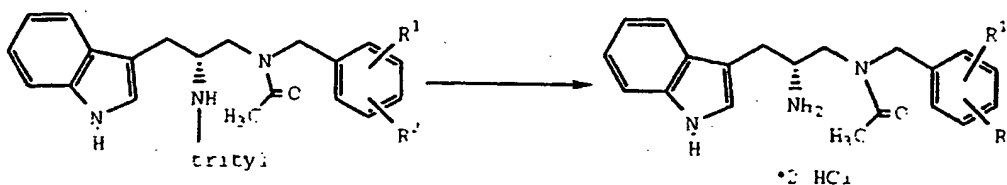


Formic acid (9.0 ml, 238.540 mmol) was added to a stirring solution of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminopropyl]-2-(N-triphenylmethylamino)propane (14.11 g, 23.763 mmol) in anhydrous methylene chloride under a nitrogen atmosphere at 0°C. After 4 hours, the reaction mixture was concentrated to an oil on a rotary evaporator and redissolved in diethyl ether and 1.0 N hydrochloric acid. The aqueous layer was washed twice with diethyl ether and basified with sodium hydroxide to a pH greater than 12. The product was extracted out with methylene chloride (4X). The organic extracts were combined, dried over anhydrous sodium sulfate, filtered, and concentrated on a rotary evaporator to a white foam. The compound (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminopropyl]propane (7.52 g, 21.397 mmols) was isolated giving a 90% yield. No further purification was necessary.

(f) Preparation of (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminopropyl]propane dihydrochloride

30

- 32 -



A stirring solution of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl-2-(N-triphenylmethylamino)propane in two volumes of methylene chloride was cooled to between -40°C and -50°C. Anhydrous hydrogen chloride gas was added at such a rate that the temperature of the reaction mixture did not exceed 0°C. The reaction mixture was stirred for 30 minutes to one hour at 0-10°C.

To this reaction mixture was added two volumes of methyl *t*-butyl ether and the resulting mixture was allowed to stir for 30 minutes to one hour at 0-10°C. The resulting crystalline solid was removed by filtration and then washed with methyl *t*-butyl ether. The reaction product was dried under vacuum at 50°C. (Yield >98%)

Analysis for $C_{21}H_{25}N_3O_2 \cdot 2 HCl$:

Theory: C, 59.44; H, 6.41; N, 9.90.

Found: C, 60.40; H, 6.60; N, 9.99.

(g) Preparation of 2-((4-cyclohexyl)piperazin-1-yl)acetic acid potassium salt hydrate

Cyclohexylpiperazine (10.0 g, 0.059 mol) was added to ten volumes of methylene chloride at room temperature. To this mixture was added sodium hydroxide (36 ml of a 2N solution, 0.072 mol) and tetrabutylammonium bromide (1.3 g, 0.004 mol). After the addition of the sodium hydroxide and tetrabutylammonium bromide, methyl bromoacetate (7.0 ml, 0.073 mol) was added and the reaction mixture was stirred for four to six hours. The progress of the reaction was monitored by gas chromatography.

- 33 -

The organic fraction was separated and the aqueous phase was back-extracted with methylene chloride. The organic phases were combined and washed twice with deionized water, once with saturated sodium bicarbonate solution, and then with brine. The organic phase was dried
5 over magnesium sulfate and the solvents were removed in vacuo to yield methyl 2-((4-cyclohexyl)piperazin-1-yl)acetate as a yellowish oil.

The title compound was prepared by dissolving
10 the methyl 2-((4-cyclohexyl)piperazin-1-yl)acetate (10.0 g, 0.042 mol) in ten volumes of diethyl ether. This solution was cooled to 15°C and then potassium trimethylsilanoate (5.9 g, 0.044) was added. This mixture was then stirred for four to six hours. The reaction product was removed by
15 filtration, washed twice with five volumes of diethyl ether, then washed twice with five volumes of hexanes, and then dried in a vacuum oven for 12-24 hours at 50°C. Analysis for $C_{12}H_{21}KN_2O_2 \cdot 1.5 H_2O$:

20	Theory:	C, 49.63; H, 7.98; N, 9.65.
	Found:	C, 49.54; H, 7.72; N, 9.11.

(h) Preparation of (R)-2-[N-(2-((4-cyclohexyl)piperazin-1-yl)acetyl)amino]-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylamino]propane

25 The title compound was prepared by first cooling 2-((4-cyclohexyl)piperazin-1-yl)acetic acid potassium salt to a temperature between -8°C and -15°C in 5 volumes of anhydrous methylene chloride. To this mixture was added
30 isobutylchloroformate at a rate such that the temperature did not exceed -8°C. The resulting reaction mixture was stirred for about 1 hour, the temperature being maintained between -8°C and -15°C.

To this mixture was then added (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylamino]propane
35 dihydrochloride at such a rate that the temperature did not

- 34 -

exceed 0°C. Next added to this mixture was N-methyl morpholine at a rate such that the temperature did not exceed 0°C. This mixture was then stirred for about 1 hour at a temperature between -15°C and -8°C.

5 The reaction was quenched by the addition of 5 volumes of water. The organic layer was washed once with a saturated sodium bicarbonate solution. The organic phase was then dried over anhydrous potassium carbonate and filtered to remove the drying agent. To the filtrate was
10 then added 2 equivalents of concentrated hydrochloric acid, followed by 1 volume of isopropyl alcohol. The methylene chloride was then exchanged with isopropyl alcohol under vacuum by distillation.

 The final volume of isopropyl alcohol was then
15 concentrated to three volumes by vacuum. The reaction mixture was cooled to 20°C to 25°C and the product was allowed to crystallize for at least one hour. The desired product was then recovered by filtration and washed with sufficient isopropyl alcohol to give a colorless filtrate.
20 The crystal cake was then dried under vacuum at 50°C.

MS 560 (M+1).

¹H NMR (CDCl₃) δ 1.09-1.28 (m, 5H), 1.64 (d, J=10 Hz, 1H), 1.80-1.89 (m, 4H), 2.10 (s, 3H), 2.24-2.52 (m, 9H), 2.90 (s, 2H), 2.95 (d, J=7 Hz, 1H), 3.02 (d, J=7 Hz, 1H), 3.12
25 (dd, J=5, 14 Hz, 1H), 3.77 (s, 3H), 4.01 (dd, J=10, 14 Hz, 1H), 4.49 (ABq, J=17 Hz, 43 Hz, 2H), 4.56 (m, 1H), 6.79-6.87 (m, 3H), 7.05-7.24 (m, 4H), 7.34-7.41 (m, 2H), 7.67 (d, J=8 Hz, 1H), 8.22 (s, 1H).

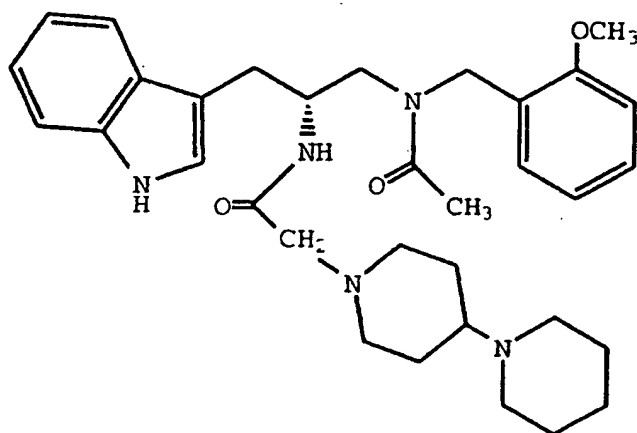
Analysis for C₃₃H₄₅N₅O₃:

30 Theory: C, 70.81; H, 8.10; N, 12.51.

Found: C, 70.71; H, 8.21; N, 12.42.

Synthesis of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl-amino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl-amino)propane
35

- 35 -



(a) Preparation of 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid, potassium salt

4-(Piperidin-1-yl)piperidine (1.20 kg, 7.13 mol) was added to methylene chloride (12.0 L) under a nitrogen atmosphere. Tetrabutylammonium bromide (0.150 kg, 0.47 mol) and sodium hydroxide (1.7 L of a 5 N solution, 8.5 mol) were then added. The reaction mixture was cooled to 10-15°C and methyl bromoacetate (1.17 kg, 7.65 mol) was added and the resulting mixture was stirred for a minimum of 16 hours.

Deionized water (1.2 L) was then added to the mixture and the layers separated. The aqueous layer was back-extracted with methylene chloride (2.4 L). The organic fractions were combined and washed with deionized water (3 x 1.2 L), a saturated sodium bicarbonate solution (1.1 L) and a saturated sodium chloride solution (1.1 L). The organic fraction was then dried over anhydrous magnesium sulfate and concentrated to an oil on a rotary evaporator to yield 1.613 kg (93.5%) of methyl 2-(4-(piperidin-1-yl)piperidin-1-yl)acetate.

A solution of methyl 2-[4-(piperidin-1-yl)piperidin-1-yl]acetate (2.395 kg, 9.96 mol) in methanol (2.4 L) was added to a solution of potassium hydroxide

- 36 -

(0.662 kg, 10.0 mol @ 85% purity) in methanol (10.5 L) under a nitrogen atmosphere. The reaction mixture was heated to 45-50°C for a minimum of 16 hours.

A solvent exchange from methanol to acetone (15.0 L) was performed on the solution on a rotary evaporator. This solution was slowly cooled to room temperature over 16 hours. The resulting solids were filtered, rinsed with acetone (5.0 L) and then dried to yield 2.471 kg (93.8%) of 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid, potassium salt.
MS 265 (M⁺¹)

(b) Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminol]propane
2-(4-(piperidin-1-yl)piperidin-1-yl)acetylaminolpropane

The title compound was prepared by first admixing (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminol]propane dihydrochloride (50.0 g, 0.118 mol) with 100 ml of methylene chloride under a nitrogen atmosphere.

In a second flask, under a nitrogen atmosphere, 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid potassium salt (62.3 g, 0.236 mol) was added to 600 ml of methylene chloride. This mixture was cooled to about -10°C and stirring was continued. To this mixture isobutylchloroformate (23 ml, 0.177 mol) was added dropwise such that the temperature of the 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid potassium salt mixture never rose appreciably.

This reaction mixture was stirred at about -10°C for about 1.5 hours at which time the (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminol]propane dihydrochloride/methylene chloride mixture prepared supra was slowly added to the 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid potassium

- 37 -

salt/isobutylchloroformate/methylene chloride solution. The resulting mixture was then stirred for about 1 hour at a temperature between -15°C and -8°C.

5 The reaction mixture was removed from the ice bath and allowed to warm to 15-20°C and the reaction was quenched by the addition of 200 ml of water. The pH of the solution was adjusted to 2.3-2.7 by the addition of 1N sulfuric acid. The layers were separated and the aqueous layer was washed with 100 ml of methylene chloride.

10 The organic fractions were combined and washed with water (100 ml). The water wash was back extracted with methylene chloride (50 ml) and combined with the aqueous fraction from above. Methylene chloride (500 ml) was added to the combined aqueous layers and the mixture
15 was stirred at room temperature for 15 minutes as basification with 2N sodium hydroxide to a final pH of 9.8 to 10.2 was achieved.

The organic and aqueous fractions were separated. The aqueous fraction was washed with methylene chloride and the
20 methylene chloride was added to the organic fraction. The organic fraction was then washed with a mixture of saturated sodium bicarbonate solution (100 ml) and water (50 ml). The bicarbonate wash was separated from the organic fraction and back extracted with methylene chloride
25 (50 ml). The back extraction was combined with the methylene chloride fraction and the combined fractions were dried over magnesium sulfate. The magnesium sulfate was removed by filtration and the volatiles were removed by vacuum distillation to yield the title product as a foam.
30 (72.5 g, >98% yield). MS 559(M+1)
NMR (DMSO-d₆ 3:2 mixture of amide rotamers) δ 1.25-1.70 (m, 10H), 1.77-2.00 (m, 2H), 1.95 (s, 3/5•3H), 2.04 (s, 2/5•3H), 2.10-2.97 (m, 9H), 3.10-3.65 (m, 3H), 3.72 (s, 2/5•3H), 3.74 (s, 3/5•3H), 4.26-4.58 (m, 3H), 6.76-7.12 (m, 6H),
35 7.13-7.35 (m, 2H), 7.42-7.66 (m, 2H), 10.80 (br s, 1H).

- 38 -

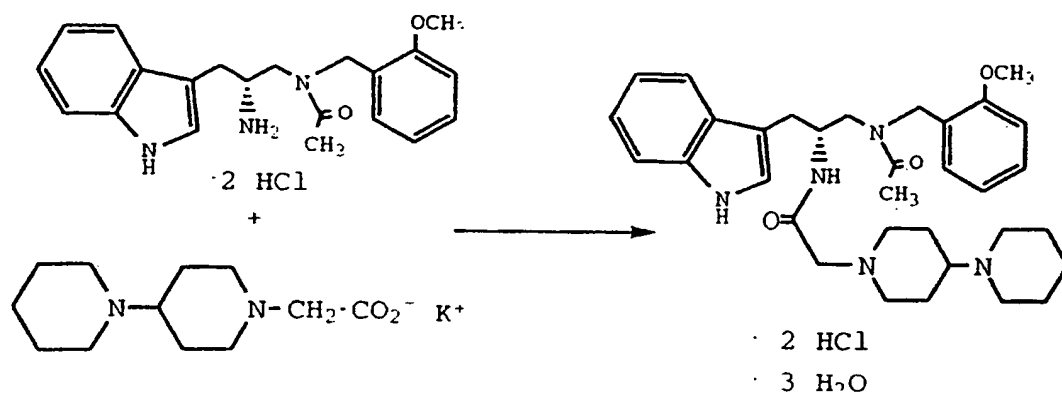
Analysis for $C_{33}H_{45}N_5O_3$:

Theory: C, 70.81; H, 8.10; N, 12.51.

Found: C, 70.57; H, 8.05; N, 12.39.

5 Alternative Synthesis

Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl]amino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane dihydrochloride
 10 trihydrate



Under a nitrogen atmosphere 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid, potassium salt (0.75 kg, 2.84 mol) was added to methylene chloride (7.5 L). The resulting mixture was cooled to -15 to -8°C and isobutyl chloroformate (0.29 kg, 2.12 mol) was added at such a rate so as to maintain the temperature of the reaction mixture below -8°C . After the addition the resulting reaction mixture was stirred for 90 minutes between -15 and -8°C .

The reaction mixture was then cooled to -35°C and solid (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)amino]propane dihydrochloride (0.60 kg, 1.14 mol) was added at such a rate that the reaction temperature was maintained at less than -20°C . After the addition, the reaction mixture was stirred for about one hour with the

- 39 -

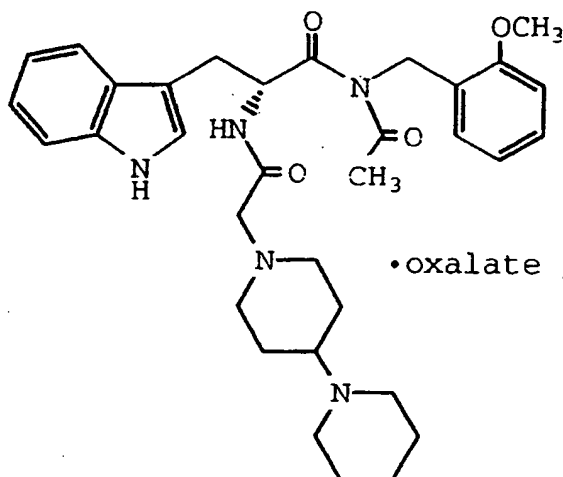
temperature being maintained between -37°C and -20°C. The reaction was quenched by the addition of deionized water (7.5 L). The reaction mixture was basified to pH 12.8-13.2 by the addition of 5 N sodium hydroxide. The aqueous
5 fraction was removed and retained. Additional deionized water (3.75 L) was added to the organic fraction as was sufficient 5 N sodium hydroxide to re-adjust the pH to 12.8-13.2.

The two aqueous fractions were combined, back-
10 extracted with methylene chloride (1.5 L) and then discarded. The organic fractions were combined and washed with deionized water (4 x 3.5 L). These extracts were combined, back-extracted with methylene chloride (1.5 L), and then discarded. The two organic layers were combined
15 and washed with a saturated sodium chloride solution (3.7 L).

The organic fraction was dried over anhydrous magnesium sulfate, filtered, and solvent exchanged from methylene chloride to acetone (3.75 L) on a rotary
20 evaporator. An aqueous solution of hydrochloric acid (0.48 L of 6 N solution, 2.88 mol) and seed crystals (2 g) were added and mixture was stirred for 30-90 minutes. Acetone (13.2 L) was then added and the slurry stirred for one hour. The resulting solid was then filtered, washed
25 with acetone (2 x 1.4 L), and dried to yield 633 g (90%) of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylamino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane dihydrochloride trihydrate.

30 Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylamino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane oxalate

- 40 -



Into a 500 ml jacketed round bottom flask was placed 2-(4-(piperidin-1-yl)piperidin-1-yl)acetic acid, potassium salt (25.0 g, 94.5 mmol) and 375 ml of N,N-dimethylformamide. The resulting slurry was cooled to -19°C and isobutylchloroformate (12.9 g, 94.5 mmol) was added over five minutes. The resulting mixture was stirred for twenty minutes and then (R)-2-amino-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetylaminopropyl]propane dihydrochloride (25.0 g, 58.1 mmol), dissolved in 75 ml of anhydrous N,N-dimethylformamide, was added over ten minutes.

The resulting mixture is then cooled to 0°C, stirred for about ten minutes, and then permitted to warm to room temperature. The progress of the reaction was monitored by chromatography. High performance liquid chromatography showed 99% conversion of the reactants after ninety minutes.

The reaction mixture was partitioned between ethyl acetate (375 ml) and a saturated sodium bicarbonate solution (375 ml). The aqueous layer was back extracted with 375 ml of ethyl acetate. The organic fractions were combined, washed with water (3 x 375 ml), and then dried over magnesium sulfate. Potassium hydroxide is then added to the aqueous fraction from above and this resulting

- 41 -

basified solution is extracted with ethyl acetate. This organic fraction is then dried over magnesium sulfate.

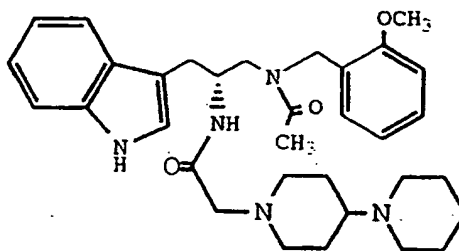
The combined dried organic fractions are then treated with a concentrated oxalic acid solution. The
5 resulting solids are filtered and dried at 50°C on a vacuum oven to yield 23.5 grams of the desired intermediate.

As would be appreciated by a skilled
10 practitioner the mixed anhydride process will work in a number of organic solvents, in addition to the anhydrous N,N-dimethylformamide depicted above. Representative examples of solvents which may be employed include acetonitrile, tetrahydrofuran, dichloromethane. The mixed
15 anhydride process can be performed at temperatures below 0°C.

The oxalate can be isolated from ethyl acetate as well as from other solvents, probably including acetone, acetonitrile, and *t*-butyl methyl ether. The use of oxalic
20 acid is, however, very important for the precipitation as a large number of acids do not give a precipitate. Among those acids attempted, but found not satisfactory for the processes of the present invention, are citric, anhydrous hydrochloric, tartaric, mandelic, trifluoroacetic, *p*-
25 nitrobenzoic, phenoxyacetic, maleic, fumaric, glutaric, adipic, methanesulfonic, *p*-toluenesulfonic, pamoic, trans-1,2-cyclohexane dicarboxylic, succinic, phthalic, trans-1,2-diaminocyclohexane-N,N,N',N'-naphthalenedisulfonic, and 5-sulfosalicylic acids. Only oxalic acid and 1,5-
30 naphthalene disulfonic acid reproducibly produced a solid.

Preparation of (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl-amino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane dihydrochloride
35 trihydrate

- 42 -



2 HCl

• 3 H₂O

Into a flask were added (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl]amino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane oxalate (3.31 g, 4.22 mmol), methylene chloride (30 ml, 39.75 g), and water (30 ml). The resulting mixture was stirred and the pH of the reaction mixture was adjusted to 10-12 using 50% caustic.

The phases were separated and the aqueous phase was extracted with methylene chloride (20 ml) and separated. The combined organic fractions were back extracted with water (30 ml) and dried over magnesium sulfate. The methylene was removed on an evaporator, leaving a residue. This residue was transferred to a jacketed flask and dissolved into acetone (24 g, 10.25 volumes).

Enough water was added to bring the water concentration to eleven percent (by weight) and the resulting mixture was heated to 55°C. Enough concentrated hydrochloric acid was added to lower the pH to 2.0 and the reaction mixture was then permitted to cool to 37°C over 45 minutes.

The product solution was seeded and permitted to stir for 10-30 minutes. The product solution was cooled to 19°C over two hours and acetone (11.8 equivalent volumes) was added over three hours, after which time the reaction mixture was stirred for one to three hours, maintaining the

- 43 -

temperature at 19°C. The product solution was filtered and the residue was washed with 10.2 equivalents (by volume) of acetone. The residue was then dried in a vacuum oven at 42°C to give the desired title product. Yield 2.407 grams (80.7%).

The biological efficacy of a compound believed to be effective as a tachykinin receptor antagonist may be confirmed by employing an initial screening assay which rapidly and accurately measured the binding of the tested compound to known NK-1 and NK-2 receptor sites. Assays useful for evaluating tachykinin receptor antagonists are well known in the art. See, e.g., J. Jukic, et al., Life Sciences, 49:1463-1469 (1991); N. Kucharczyk, et al., Journal of Medicinal Chemistry, 36:1654-1661 (1993); N. Rouissi, et al., Biochemical and Biophysical Research Communications, 176:894-901 (1991).

NK-1 Receptor Binding Assay

Radioreceptor binding assays were performed using a derivative of a previously published protocol. D.G. Payan, et al., Journal of Immunology, 133:3260-3265 (1984). In this assay an aliquot of IM9 cells (1×10^6 cells/tube in RPMI 1604 medium supplemented with 10% fetal calf serum) was incubated with 20 pM ^{125}I -labeled substance P in the presence of increasing competitor concentrations for 45 minutes at 4°C.

The IM9 cell line is a well-characterized cell line which is readily available to the public. See, e.g., Annals of the New York Academy of Science, 190: 221-234 (1972); Nature (London), 251:443-444 (1974); Proceedings of the National Academy of Sciences (USA), 71:84-88 (1974). These cells were routinely cultured in RPMI 1640

- 44 -

supplemented with 50 µg/ml gentamicin sulfate and 10% fetal calf serum.

The reaction was terminated by filtration through a glass fiber filter harvesting system using
5 filters previously soaked for 20 minutes in 0.1% polyethylenimine. Specific binding of labeled substance P was determined in the presence of 20 nM unlabeled ligand.

Many of the compounds employed in the methods of
10 the present invention are also effective antagonists of the NK-2 receptor.

NK-2 Receptor Binding Assay

15 The CHO-hNK-2R cells, a CHO-derived cell line transformed with the human NK-2 receptor, expressing about 400,000 such receptors per cell, were grown in 75 cm² flasks or roller bottles in minimal essential medium (alpha modification) with 10% fetal bovine serum. The gene
20 sequence of the human NK-2 receptor is given in N.P. Gerard, et al., Journal of Biological Chemistry, 265:20455-20462 (1990).

For preparation of membranes, 30 confluent roller bottle cultures were dissociated by washing each
25 roller bottle with 10 ml of Dulbecco's phosphate buffered saline (PBS) without calcium and magnesium, followed by addition of 10 ml of enzyme-free cell dissociation solution (PBS-based, from Specialty Media, Inc.). After an additional 15 minutes, the dissociated cells were pooled
30 and centrifuged at 1,000 RPM for 10 minutes in a clinical centrifuge. Membranes were prepared by homogenization of the cell pellets in 300 ml 50 mM Tris buffer, pH 7.4 with a Tekmar® homogenizer for 10-15 seconds, followed by centrifugation at 12,000 RPM (20,000 x g) for 30 minutes
35 using a Beckman JA-14® rotor. The pellets were washed once using the above procedure. and the final pellets were

- 45 -

resuspended in 100-120 ml 50 mM Tris buffer, pH 7.4, and 4 ml aliquots stored frozen at -70°C. The protein concentration of this preparation was 2 mg/ml.

For the receptor binding assay, one 4-ml aliquot
5 of the CHO-hNK-2R membrane preparation was suspended in 40 ml of assay buffer containing 50 mM Tris, pH 7.4, 3 mM manganese chloride, 0.02% bovine serum albumin (BSA) and 4 µg/ml chymostatin. A 200 µl volume of the homogenate (40 µg protein) was used per sample. The radioactive ligand
10 was [¹²⁵I]iodohistidyl-neurokinin A (New England Nuclear, NEX-252), 2200 Ci/mmol. The ligand was prepared in assay buffer at 20 nCi per 100 µl; the final concentration in the assay was 20 pM. Non-specific binding was determined using 1 µM eledoisin. Ten concentrations of eledoisin from
15 0.1 to 1000 nM were used for a standard concentration-response curve.

All samples and standards were added to the incubation in 10 µl dimethylsulfoxide (DMSO) for screening (single dose) or in 5 µl DMSO for IC₅₀ determinations. The
20 order of additions for incubation was 190 or 195 µl assay buffer, 200 µl homogenate, 10 or 5 µl sample in DMSO, 100 µl radioactive ligand. The samples were incubated 1 hr at room temperature and then filtered on a cell harvester through filters which had been presoaked for two hours in
25 50 mM Tris buffer, pH 7.7, containing 0.5% BSA. The filter was washed 3 times with approximately 3 ml of cold 50 mM Tris buffer, pH 7.7. The filter circles were then punched into 12 x 75 mm polystyrene tubes and counted in a gamma counter.

30

Animal and human clinical models demonstrating the effectiveness of the methods of the present invention are well known to those skilled in the art. For example, in evaluating the methods of the present invention in
35 treating or preventing anxiety the following models may be employed.

- 46 -

Punished Responding

The antianxiety activity of the compositions employed in the method of the present invention is established by demonstrating that these compositions increase punished responding. This procedure has been used to establish antianxiety activity in clinically established compositions.

According to this procedure, the responding of rats or pigeons is maintained by a multiple schedule of food presentation. In one component of the schedule, responding produces food pellet presentation only. In a second component, responding produces both food pellet presentation and is also punished by presentation of a brief electric shock. Each component of the multiple schedule is approximately 4 minutes in duration, and the shock duration is approximately 0.3 seconds. The shock intensity is adjusted for each individual animal so that the rate of punished responding is approximately 15 to 30% of the rate in the unpunished component of the multiple schedule. Sessions are conducted each weekday and are approximately 60 min in duration. Vehicle or a dose of composition are administered 30 min to 6 hours before the start of the test session by the subcutaneous or oral route. Composition effects for each dose for each animal are calculated as a percent of the vehicle control data for that animal. The data are expressed as the mean \pm the standard error of the mean.

Monkey Taming Model

The antianxiety activity of the compositions is established by demonstrating that the compositions are effective in the monkey taming model. Plotnikoff, Res. Comm. Chem. Path. & Pharmacol., 5:128-134 (1973) describes

- 47 -

the response of rhesus monkeys to pole prodding as a method of evaluating the antiaggressive activity of a test composition. In this method, the antiaggressive activity of a composition is considered to be indicative of its antianxiety activity. Hypoactivity and ataxia are considered to be indicative of a sedative component of the composition. The present study is designed to measure the pole prod response-inhibition induced by a composition of this invention in comparison with that of a standard antianxiety composition employing a compound such as diazepam as a measure of antiaggressive potential, and to obtain an indication of the duration of action of the compound.

Male and female rhesus or cynomologous monkeys, selected for their aggressiveness toward a pole, are housed individually in a primate colony room. Compositions or appropriate vehicle are administered orally or subcutaneously and the animals are observed by a trained observer at varying times after drug administration. A minimum of three days (usually a week or more) elapses between treatments. Treatments are assigned in random fashion except that no monkey receives the same composition two times consecutively.

Aggressiveness and motor impairment are graded by response to a pole being introduced into the cage as described in Table II. The individuals responsible for grading the responses are unaware of the dose levels received by the monkeys.

- 48 -

Table II

Grading of Monkey Response to Pole Introduction

	<u>Response</u>	<u>Grade</u>	<u>Description</u>
5	Attack	2	Monkey immediately grabbed and/or bit pole as it was placed at opening in cage.
		1	Monkey grabbed and/or bit pole only after the tip was extended into the cage 12 inches or more.
10		0	No grabbing or biting observed.
	Pole Push	2	Monkey grabbed the pole to attack it or push it away.
15		1	Monkey touched the pole only in attempting to avoid it or rode on the pole (avoidance).
		0	No pushing, grabbing or riding of the pole observed.
20	Biting	2	Monkey bit aggressively and frequently.
		1	Monkey bit weakly or infrequently.
		0	No biting observed.
	Ataxia	2	Monkey exhibited a marked loss of coordination.
25		1	Slight loss of coordination observed.
		0	No effects on coordination observed.
	Hypoactivity	2	Marked: Monkey was observed in a prone position. May or may not have responded by rising and moving away when experimenter approached.
30		1	Slight: Monkey did not retreat as readily when experimenter approached.
35		0	None.

- 49 -

Antiaggression +	Dose of drug was active in decreasing
Activity of	global assessment of aggressive behavior
Drug Dose -	Dose of drug was not active in
	decreasing aggressive behavior

5

Human Clinical Trials

Finally, the antianxiety activity of the named compositions and methods can be demonstrated by human clinical trials. The study is designed as a double-blind, parallel, placebo-controlled multicenter trial. The patients are randomized into four groups, placebo and 25, 50, and 75 mg tid of test composition. The dosages are administered orally with food. Patients are observed at four visits to provide baseline measurements. Visits 5-33 served as the treatment phase for the study.

During the visits, patients and their caregivers were questioned and observed for signs of agitation, mood swings, vocal outbursts, suspiciousness, and fearfulness. Each of these behaviors are indicative of the effect of the test composition on an anxiety disorder.

Numerous recent publications have demonstrated that migraine and numerous psychiatric disorders are comorbid. Individuals with migraine are at a higher risk of developing these disorders, which are described in detail infra. N. Breslau, et al., Headache, 34:387-393 (1994); K.R. Merikangas, et al., Archives of General Psychiatry, 47:849-853 (1990); N. Breslau, et al., Psychiatry Research, 37:11-23 (1991); W.F. Stewart, et al., Psychosom. Medicine, 51:559-569; J. Jarman, et al., Journal of Neurological and Neurosurgical Psychiatry, 53:573-575 (1990); V. Glover, et al., Journal of Psychiatric Research, 27:223-231 (1993); N. Breslau and G.C. Davis, Journal of Psychiatric Research, 27:211-221 (1993); and K.R. Merikangas, et al., Journal of Psychiatric Research, 27:197-210 (1993).

- 50 -

Co-pending United States Patent Application 08/318,330, filed October 5, 1994, clearly demonstrates that those compounds having an affinity for the 5-HT_{1F} receptor subtype are most advantageous for the treatment of migraine. Co-pending United States Patent Application Serial Number 08/318,391, filed October 5, 1994, clearly demonstrates that a combination of a serotonin agonist and a tachykinin receptor antagonist are superior to either class of compound alone in the treatment of migraine, the combination demonstrating a synergistic efficacy profile.

The advantages of any synergistic combination therapy are obvious. Among its other advantages, this combination therapy greatly increases the therapeutic index of a composition in treating these psychiatric disorders. A markedly decreased amount of a serotonin agonist may now be administered to a patient, presumably greatly lessening the likelihood and severity of any adverse events. The reduced amount of active ingredient necessary for a therapeutic effect makes possible other routes of formulation than those currently employed. Rapid onset formulations such as buccal or sublingual may now be developed. Sustained release formulations are now more feasible due to the lower amounts of active ingredient necessary.

While it is possible to administer a compound employed in the methods of this invention directly without any formulation, the compounds are usually administered in the form of pharmaceutical compositions comprising a pharmaceutically acceptable excipient and at least one active ingredient. These compositions can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Many of the compounds employed in the methods of this invention are effective as both injectable and oral compositions. Such compositions are prepared in a manner

- 51 -

well known in the pharmaceutical art and comprise at least one active compound. See, e.g., REMINGTON'S PHARMACEUTICAL SCIENCES, (16th ed. 1980).

In making the compositions employed in the present invention the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and

- 52 -

propylhydroxybenzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 to about 100 mg, more usually about 1.0 to about 30 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The active compounds are generally effective over a wide dosage range. For examples, dosages per day normally fall within the range of about 0.01 to about 30 mg/kg of body weight. In the treatment of adult humans, the range of about 0.1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound or compounds administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

- 53 -

Formulation Preparation 1

Hard gelatin capsules containing the following ingredients are prepared:

5

<u>Ingredient</u>		<u>Quantity</u> <u>(mg/capsule)</u>
Active Ingredient(s)		30.0
10	Starch	305.0
	Magnesium stearate	5.0

The above ingredients are mixed and filled into
15 hard gelatin capsules in 340 mg quantities.

- 54 -

Formulation Preparation 2

A tablet formula is prepared using the ingredients below:

5		Quantity
	<u>Ingredient</u>	<u>(mg/tablet)</u>
	Active Ingredient(s)	25.0
10	Cellulose, microcrystalline	200.0
	Colloidal silicon dioxide	10.0
	Stearic acid	5.0

15

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Preparation 3

20

A dry powder inhaler formulation is prepared containing the following components:

	<u>Ingredient</u>	<u>Weight %</u>
25	Active Ingredient(s)	5
	Lactose	95

The active mixture is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

30

- 55 -

Formulation Preparation 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

5		
	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
	Active Ingredient(s)	30.0 mg
10	Starch	45.0 mg
	Microcrystalline cellulose	35.0 mg
	Polyvinylpyrrolidone	
15	(as 10% solution in water)	4.0 mg
	Sodium carboxymethyl starch	4.5 mg
	Magnesium stearate	0.5 mg
20	Talc	<u>1.0 mg</u>
	Total	120 mg

25 The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 30 50-60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each 35 weighing 120 mg.

- 56 -

Formulation Preparation 5

Capsules, each containing 40 mg of medicament are made as follows:

5		Quantity
	<u>Ingredient</u>	<u>(mg/capsule)</u>
	Active Ingredient(s)	40.0 mg
10	Starch	109.0 mg
	Magnesium stearate	<u>1.0 mg</u>
	Total	150.0 mg

15

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

20

Formulation Preparation 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

25

	<u>Ingredient</u>	<u>Amount</u>
	Active Ingredient(s)	25 mg
	Saturated fatty acid glycerides to	2,000 mg

30

The active ingredient(s) is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

35

- 57 -

Formulation Preparation 7

Suspensions, each containing 50 mg of medicament
5 per 5.0 ml dose are made as follows:

	<u>Ingredient</u>	<u>Amount</u>
	Active Ingredient(s)	50.0 mg
10	Xanthan gum	4.0 mg
	Sodium carboxymethyl cellulose (11%)	
	Microcrystalline cellulose (89%)	50.0 mg
15	Sucrose	1.75 g
	Sodium benzoate	10.0 mg
	Flavor and Color	q.v.
20	Purified water to	5.0 ml

The medicament, sucrose and xanthan gum are
blended, passed through a No. 10 mesh U.S. sieve, and then
25 mixed with a previously made solution of the
microcrystalline cellulose and sodium carboxymethyl
cellulose in water. The sodium benzoate, flavor, and color
are diluted with some of the water and added with stirring.
Sufficient water is then added to produce the required
30 volume.

- 58 -

Formulation Preparation 8

Capsules, each containing 15 mg of medicament,
5 are made as follows:

	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient(s)	15.0 mg
10	Starch	407.0 mg
	Magnesium stearate	<u>3.0 mg</u>
15	Total	425.0 mg

The active ingredient(s), cellulose, starch, and
magnesium stearate are blended, passed through a No. 20
mesh U.S. sieve, and filled into hard gelatin capsules in
20 425 mg quantities.

Formulation Preparation 9

An intravenous formulation may be prepared as
25 follows:

	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient(s)	250.0 mg
30	Isotonic saline	1000 ml

- 59 -

Formulation Preparation 10

A topical formulation may be prepared as follows:

5

<u>Ingredient</u>	<u>Quantity</u>
Active Ingredient(s)	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

10

15 The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

20

- 60 -

Formulation Preparation 11

Sublingual or buccal tablets, each containing 10 mg of active ingredient, may be prepared as follows:

5		Quantity
	<u>Ingredient</u>	<u>Per Tablet</u>
	Active Ingredient(s)	10.0 mg
10	Glycerol	210.5 mg
	Water	143.0 mg
	Sodium Citrate	4.5 mg
15	Polyvinyl Alcohol	26.5 mg
	Polyvinylpyrrolidone	<u>15.5 mg</u>
	Total	410.0 mg

20

The glycerol, water, sodium citrate, polyvinyl alcohol, and polyvinylpyrrolidone are admixed together by continuous stirring and maintaining the temperature at about 90°C. When the polymers have gone into solution, the solution is cooled to about 50-55°C and the medicament is slowly admixed. The homogenous mixture is poured into forms made of an inert material to produce a drug-containing diffusion matrix having a thickness of about 2-4 mm. This diffusion matrix is then cut to form individual tablets having the appropriate size.

30

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled

35

- 61 -

amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may
5 be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually
10 involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent 5,011,472,
15 issued April 30, 1991, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of
20 hydrophilic drugs into lipid-soluble drugs or prodrugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain
25 barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

30 The type of formulation employed for the administration of the compounds employed in the methods of the present invention may be dictated by the particular compounds employed, the type of pharmacokinetic profile desired from the route of administration and the
35 compound(s), and the state of the patient.

- 62 -

The administration of the serotonin agonist may be simultaneous with, before, or after the administration of the tachykinin receptor antagonist. If it is desired to administer the serotonin agonist simultaneously with the tachykinin receptor antagonist, the two active ingredients may be combined into one pharmaceutical formulation or two formulations may be administered to the patient.

Many factors will dictate the order of administration of the serotonin agonist and the tachykinin receptor antagonist. Some of these considerations include: the particular compounds employed; the manner in which each active ingredient is formulated; whether the administration is prophylactic or curative in nature; and the condition of the patient.

The patient to be benefited by practice of the present invention is a patient having one or more of the disorders discussed in detail below, or who is at a heightened risk of contracting such disorder. Diagnosis of these disorders, or the identification of a patient at risk of one or more of them, is to be made by a physician or psychiatrist. It is presently believed that the combination of serotonin receptor agonists and tachykinin receptor antagonists results in the alleviation of the effects of the disorder from which the patient suffers, or even the elimination of the disorder completely.

A patient with a heightened risk of contracting one of the present disorders is a patient, in the present contemplation, who is more likely than is a normal person to fall victim to that disorder. The patient may have suffered from the disorder in the past, and be at risk of a relapse, or may exhibit symptoms which demonstrate to the physician or psychiatrist that the patient is under an abnormal risk of developing the disorder in its full form.

- 63 -

The disorders which are treated or prevented in the practice of the present invention may be described as follows.

5 bulimia nervosa
obsessive-compulsive disorder
premenstrual dysphoric disorder
substance abuse
substance dependence
10 panic disorder
panic attack
agoraphobia
post-traumatic stress disorder
dementia of Alzheimer's type
15 social phobia
attention deficit hyperactivity disorder
disruptive behavior disorder
intermittent explosive disorder
borderline personality disorder
20 chronic fatigue syndrome
premature ejaculation
depression and behavioral problems associated
with head injury, mental retardation or stroke.

25 Most of the disorders discussed here are described and categorized in the DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS, (4th edition, 1994), published by the American Psychiatric Association (hereinafter referred to as DSM). In the discussion below, the DSM codes for the
30 disorders will be given where appropriate.

Bulimia nervosa, DSM 307.51, is characterized by uncontrollable binge eating, followed by self-induced purging, usually vomiting. Its prevalence is as high as 1%-3% among adolescent and young adult females. The
35 disorder is well characterized and recognized by the health professions. The essential features of it are binge eating

- 64 -

and inappropriate compensatory methods to prevent weight gain. Further, individuals with the disorder are excessively influenced by body shape and weight.

Obsessive-compulsive disorder, DSM 300.3, is
5 characterized by recurrent obsessions or compulsions which are severe enough to be time consuming or cause distress or impairment of the patient's life. Obsessions are
persistent ideas, thoughts, impulses or images which are recognized by the patient to be intrusive and inappropriate
10 and cause anxiety or distress. The individual senses that the obsession is alien, not under control and not the kind of thought that the patient would expect to have. Common obsessions include repeated thoughts about contamination, repeated doubts, a need to arrange things in a particular
15 order, aggressive or horrific impulses and sexual imagery. Compulsions are repetitive behaviors, such as hand washing, or mental acts, such as counting or repeating words silently, the goal of which is to prevent or reduce anxiety or distress. By definition, compulsions are either clearly
20 excessive or not realistically connected with that which they are designed to neutralize or prevent. Obsessive-compulsive disorder is rather common, with an estimated lifetime prevalence of 2.5%.

Substance abuse and substance dependence, very
25 well known in most societies at present, come about when the patient becomes addicted or habituated to the improper use of a drug or other substance. Several different varieties of substance abuse and dependence will be discussed in detail below. It will be understood that
30 substance abuse or dependence often results in additional disorders, including intoxication, withdrawal symptoms, delirium, psychotic disorders, hallucinations, mood disorders, anxiety disorders, sexual dysfunctions, or sleep disorders. Recognized substance abuse and substance
35 dependence disorders which are part of the present invention include the following:

- 65 -

amphetamine dependence, DSM 304.40
amphetamine abuse, DSM 305.70
cannabis dependence, DSM 304.30
5 cannabis abuse, DSM 305.20
cocaine dependence, DSM 304.20
cocaine abuse, DSM 305.60
hallucinogen dependence, DSM 304.50
hallucinogen abuse, DSM 305.30
10 inhalant dependence, DSM 304.60
inhalant abuse, DSM 305.90
nicotine dependence, DSM 305.10
opioid dependence, DSM 304.00
opioid abuse, DSM 305.50
15 phencyclidine dependence, DSM 304.90
phencyclidine abuse, DSM 305.90
sedative, hypnotic or anxiolytic dependence, DSM
304.10
sedative, hypnotic or anxiolytic abuse, DSM 305.40
20 polysubstance dependence, DSM 304.80

The prevalence and deleterious effects of
substance dependence and substance abuse are almost too
well known to discuss. The disorders are characterized, in
25 general, by a compulsion to use the substance in question
in order to obtain its effects, regardless of the ill-
effects of the substance or the difficulty, expense or
danger of obtaining it. Some substances of abuse, such as
cannabis and cocaine, have run through entire sections of
30 society and have damaged or ruined untold numbers of lives.
The importance of duloxetine's ability to relieve such
disorders in accordance with the present invention is
obviously of great significance.

Panic attack, panic disorder and agoraphobia,
35 categorized as DSM 300.01, 300.21 and 300.22, affect
between 1.5% and 3.5% of the population. The disorders are

- 66 -

characterized by irrational sense of imminent danger or doom, an urge to escape, or a fear of being in a situation from which escape might be difficult. The patient exhibits symptoms such as palpitations, accelerated heart rate, 5 sweating, sensations of shortness of breath, chest pain, nausea, dizziness, fear of dying, and the like, and may have such attacks very frequently.

Social phobia, DSM 300.23, produces a marked and persistent fear of social or performance situations in 10 which embarrassment may occur. Exposure to such a situation may result in a panic attack, or other anxious response. Most often, patients with the disorder simply avoid situations of the type which they dread, producing an obvious dislocation in the patient's life. The prevalence 15 of social phobia has been reported as from 3% to 13%, on a lifetime basis.

Post-traumatic stress disorder, DSM 309.81, afflicts patients following exposure to a traumatic stress involving personal experience of an event involving actual 20 or threatened death of injury. Such traumatic events include experiences such as military combat, personal assault, kidnapping, terrorist attack, torture, natural or man-made disasters, severe accidents, or being diagnosed with a dreaded illness. Learning about such events 25 occurring to others, particularly a family member or close friend, also may produce the disorder. Triggering events which symbolize the traumatic event, such as an anniversary, may recreate the stress and bring on the disorder long after the event is passed. Patients strive 30 to avoid stimuli associated with the trauma, even to the point of amnesia or reduced responsiveness to other people in general. Prevalence of post-traumatic stress disorder has been reported at from 1% to as much as 14%, and has been reported at 50% and more in studies of individuals who 35 are at risk of the disorder.

- 67 -

Dementia of the Alzheimer's type, DSM 290.11, 290.12, 290.13, 290.10, 290.3, 290.20, 290.21 and 290.0, affects between 2% and 4% of the population over 65 years old. The prevalence increases with age, particularly after
5 75 years of age, and is associated with Alzheimer's disease. In most patients, brain atrophy or deterioration is present, and is associated with the dementia.

Attention deficit hyperactivity disorder, DSM 314.01 and 314.00, is primarily recognized as a disorder of
10 children, but may well be found in adults as well. It is characterized by symptoms such as lack of attention, impulsivity, and excessive activity, resulting in high expenditure of effort accompanied with a low degree of accomplishment. Patients have difficulty or find it
15 impossible to give attention to details, cannot sustain attention in tasks or even play, and make careless mistakes. They fail to listen to or follow through on instructions, lose things, and are easily distracted by extraneous events. The difficulty of such patients in
20 carrying out useful lives is obvious from the mere recital of the symptoms.

Disruptive behavior disorder, DSM 312.9, is a condition characterized by aggressive, destructive, deceitful and defiant activity.

25 Intermittent explosive disorder, DSM 312.34, is characterized by episodes of failure to resist aggressive impulses, resulting in assault or destruction of property. The degree of aggressiveness expressed during episodes of this disorder is grossly disproportionate to any
30 provocation or triggering stress. The Southeastern Asian condition of amok is an episode of this disorder, cases of which have been reported in Canada and the United States as well.

Borderline personality disorder, DSM 301.83, is
35 marked by a pervasive pattern of instability of interpersonal relationships and self-image, and marked

- 68 -

impulsivity which begins by early adulthood. Patients have a pattern of unstable and intense relationships, very quickly developing a very close relationship and then quickly devaluing the other person. Patients may gamble, spend irresponsibly, binge eat, abuse substances, engage in unsafe sex or drive recklessly. Patients often display recurrent suicidal behavior or self-injurious behavior. The prevalence is estimated to be about 2% of the population.

10 Premature ejaculation, DSM 302.75, is characterized by the inability of a male to delay orgasm as long as is desired.

Depression and behavioral problems associated with head injury, mental retardation or stroke are treated in the exercise of the present invention. Such depression and behavioral problems are distinct from the usual such disorders, because of their origin. Depression, of course, of the general type is quite prevalent and is now well-known, being well treated with pharmaceuticals such as, for example, fluoxetine.

Chronic fatigue syndrome is a condition which has been variously described and diagnosed. It is sometimes categorized as a low-grade viral infection, particularly caused by the Epstein-Barr virus. Since that virus is very widely found in the population, however, the diagnosis is problematic. An alternative characterization of chronic fatigue syndrome is a physical-psychological disorder of the depression type, characterized primarily by lack of energy and listlessness.

30 Premenstrual dysphoric disorder is characterized by symptoms such as feelings of sadness, hopelessness or self-deprecation; anxiety or tenseness; tearfulness and lability of mood; persistent irritability and anger; decreased interest in usual activities or withdrawal from relationships; difficulty concentrating and the like. It is not classified formally by DSM but is discussed in

- 69 -

detail there. The pattern of symptoms occurs in most cycles, frequently beginning the week prior to menses. Frequently, the disorder markedly interferes with the patient's life in all respects during the attack of the

5 disorder. The prevalence of the disorder in its most profound form has been estimated at 3%-5%, but there has been little systematic study on the course and stability of the condition.

- 70 -

We Claim:

1. A method for the treatment or prevention of a psychiatric disorder in a mammal which comprises
5 administering to a mammal in need thereof an effective amount of a combination of a tachykinin receptor antagonist and a serotonin agonist.
2. A method as claimed in Claim 1 in which the
10 mammal in need of treatment is administered a composition possessing both tachykinin receptor antagonist activity and serotonin agonist activity.
3. A method for the treatment or prevention of
15 a psychiatric disorder in a mammal which comprises administering to a mammal in need thereof a composition possessing serotonin agonist activity followed by a composition possessing tachykinin receptor antagonist activity.
20
4. A method for the treatment or prevention of a psychiatric disorder in a mammal which comprises
administering to a mammal in need thereof an effective
amount of a combination of a tachykinin receptor antagonist
25 and a selective serotonin reuptake inhibitor.
5. A pharmaceutical formulation for use in
treating a psychiatric disorder in a mammal which comprises
admixing a compound having activity as a tachykinin
30 receptor antagonist with a compound having activity as a serotonin agonist.
6. A pharmaceutical formulation for use in
treating a psychiatric disorder in a mammal which comprises
35 admixing a compound having activity as a tachykinin

- 71 -

receptor antagonist with a compound having activity as a selective serotonin reuptake inhibitor.

7. A method as claimed in any one of Claims 1 to 4 in which the serotonin agonist has affinity for the 5-HT_{1F} receptor subtype.

8. A method as claimed in Claim 7 in which the serotonin agonist is sumatriptan, (S)-4-[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]-2-oxazolidinone, or 5-fluoro-3-[1-[2-[1-methyl-1H-pyrazol-4-yl]ethyl]-4-piperidinyl]-1H-indole hydrochloride.

9. A method as claimed in any one of Claims 1 to 4 in which the tachykinin receptor antagonist is (R)-2-[N-(2-((4-cyclohexyl)piperazin-1-yl)acetyl)amino]-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl]amino]propane, (R)-3-(1H-indol-3-yl)-1-[N-(2-methoxybenzyl)acetyl]amino]-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane, 1-(2-bromobenzyl)-2-(3,5-dimethylphenyl)-6-[2-(N,N-dimethylamino)ethoxy]benzimidazole, RP 67580, (±)CP 96345, 5-(3,5-bistrifluoromethylphenyl)-1-(3-indolyl)-2-((4-methylpiperazin-1-yl)acetamido)-3-pentanone, (4-methylphenyl)methyl [R-(R*,S*)]-[1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[(1-phenylethyl)amino]ethyl]carbamate, or 1-(3,5-dimethylbenzyloxy)-2-amino-2-phenylcyclohexane, or a salt or solvate thereof.

10. A method as claimed in any one of Claims 1 to 4 wherein the psychiatric disorder is selected from the group consisting of panic disorder, panic attack, depression, anxiety, bulimia nervosa, obsessive-compulsive disorder, premenstrual dysphoric disorder, substance abuse, substance dependence, agoraphobia, post-traumatic stress disorder, dementia of Alzheimer's type, social phobia, attention deficit hyperactivity disorder, disruptive

- 72 -

behavior disorder, intermittent explosive disorder,
borderline personality disorder, chronic fatigue syndrome,
premature ejaculation, and depression and behavioral
problems associated with head injury, mental retardation or
5 stroke.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01737**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A61K 31/445, 31/405.

US CL : 514/327, 329, 415.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/327, 329, 415.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

TERMS SEARCHED: SEROTONIN AGONIST AND ALZHEIMER? AND TACHYDININ ANTAGONIST.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,296,497 (HARTOG ET AL.) 22 MARCH 1994, COLUMN 5, LINE 10 TO COLUMN 6, LINE 35.	1-10
Y	US, A, 5,444,074 (BAKER ET AL.) 22 AUGUST 1995, COLUMN 1, LINE 10 TO COLUMN 2, LINE 24.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 24 MAY 1996	Date of mailing of the international search report 31 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THEODORE J. CRIARES Telephone No. (703) 308-1235